Amendments To The Specification

Please amend the specification and abstract as follows:

The paragraph on page 2 beginning at line 5,

Fort Dodge Animal Health (FDAH), markets *Mycoplasma hyopneumoniae* bacterin under the name Suvaxyn® Respifend® MH for use as a vaccine to protect healthy swine against clinical signs caused by *Mycoplasma hyopneumoniae*. The vaccine contains CARBOPOL as an adjuvant and is recommended as a two-dose vaccine for one-week old pigs, with the second dose two to three weeks after the first vaccination. However, a two-dose vaccine has the obvious disadvantage of requiring a second handling of the animals in order to provide full protection against disease.

The paragraph on page 3 beginning at line 4,

In another aspect, the present invention provides an immunogenic composition for immunizing an animal against infection by *Mycoplasma hyopneumoniae*, comprising an inactivated *Mycoplasma hyopneumoniae* bacterin combined with the above adjuvant mixture and a pharmaceutically acceptable stabilizer, carrier or diluent. The adjuvant is usually present in this vaccine composition at a final concentration of about 1-25% (v/v), and preferably about 5-12% (v/v). The composition may also include other vaccine components, including inactivated bacterins or purified toxoids, from one or more pathogens, such as ~~*Haemonphilus*~~ *Haemophilus parasuis, Pasteurella multocida*~~*multiocida*~~, ~~*Streptococcum*~~ *Streptococcus suis, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Salmonella choleraesuis* and leptospira and may be administered by intramuscular, subcutaneous, oral, aerosol or intranasal routes.

The paragraph on pages of 4 and 5 beginning at line 26 of page 4,

The adjuvant mixture for use in the vaccine compositions of the present invention enhances the immune response and comprises a mixture of an acrylic acid polymer with a mixture of metabolizable oil, *e.g.*, an unsaturated terpene hydrocarbon or a hydrogenation product thereof, preferably squalane (2,3,10,15,19,23-hexamethyltetracosane) or squalene, and a polyoxyethylene-polyoxypropylene block copolymer. Such an acrylic acid polymer may be a homopolymer or a copolymer. The acrylic acid polymer is preferably a carbomer. Carbomers are commercially available under the trade name ~~Carbopol~~CARBOPOL. Acrylic

acid polymers are described, for example, in U. S. Patents 2,909,462 and 3,790,665, whose disclosures are incorporated herein by reference. The polyoxyethylene-polyoxypropylene block copolymers are surfactants, preferably liquid surfactants that aid in suspending solid and liquid components. The surfactants are commercially available as polymers under the trade name ~~Pluronic~~PLURONIC®. The preferred surfactant is poloxamer 401 which is commercially available under the trade name ~~Pluronic~~PLURONIC® L121.

The paragraph on page 5 beginning at line 17,

In this adjuvant mixture, the metabolizable oil and the acrylic acid polymer may be present in amounts ranging from about 10 to 150 ml/L and about 0.5 to 10g/L, respectively. In a preferred embodiment of the adjuvant mixture, the mixture of the metabolizable oil and polyoxyethylene-polyoxypropylene block copolymer component is a mixture of squalane and ~~Pluronic~~PLURONIC® L121 (poloxamer 401) which may be present in an amount of about 50 to 100 ml/L and the carboxymethylene polymer is ~~Carbopol~~CARBOPOL 934P (Carbamer 934P) which may be present in amount of about 2 ml/L. Typically, the adjuvant mixture contains about a 1:25 to 1:50 ratio of acrylic acid polymer to metabolizable oil/ polyoxyethylene-polypropylene block copolymer mixture.

The paragraph on page 5 beginning at line 27,

Preferred polyacrylic acid polymers are those marketed by B. F Goodrich as ~~Carbopol~~CARBOPOL 934 P NF and 941 NF which are polymers of acrylic acid cross-linked with polyallylsucrose and which have the chemical formula $(CH_2CHOOOH)_n$. These polymers form aqueous gels which suitably formulate with aqueous carriers. Preferred polyoxyethylene-polypropylene block copolymers are the nonionic surfactants marketed by BASF as ~~Pluronic~~PLURONIC® L121, L61, L,81 or L101.

The paragraph on page 7 beginning at line 20,

A mixture of a metabolizable oil that comprises one or more terpene hydrocarbons and a polyoxyethylene-polypropylene block copolymer, *e.g.*, a Squalane/~~Pluronic~~PLURONIC® L121 mixture, is prepared by dissolving 10 g. sodium chloride, 0.25 g potassium chloride, 2.72 sodium phosphate dibasic, 0.25 potassium phosphate monobasic, 20 mL ~~Pluronic~~PLURONIC® L121 (BASF Corporation), 40 mL Squalane (Kodak), 3.2 mL ~~Tween~~TWEEN 80 in 900 mL purified water, q.s. to 1000 ml. After mixing, the ingredients

may be autoclaved. The mixture is then homogenized until a stable emulsion is formed. Formalin may be added up to a final concentration of 0.2% or thimerosal may be added to a final concentration of 1:10,000.

The table on page 8,

Amounts for 1,000,000 doses (2mL each):		% vol/vol
Mycoplasma Concentrate (>1.0 x10^{10} MHDCE/mL)	400,000 mL	20.0
Squalane/~~Pluronic~~PLURONIC® L121 mixture	100,000 mL	5.0
~~Carbopol~~CARBOPOL (2% w/v in water)	200,000 mL	10.0
Thimerosal Solution 1% w/v in water and EDTA (tetrasodium salt) 7 w/v%	18,000 mL	0.9
Sterile Saline	1,282,000 mL	64.1

The pH of the serial is adjusted to 7.0 ± 0.2.

MHDCE = *Mycoplasma hyopneumoniae* DNA Cell equivalents

The table on page 9 starting at line 18,

Ingredients	Liter
NaCl	8.50 grams
NaH_2PO_4	0.22 grams
Na_2HPO_4	1.19 grams
~~Tween~~TWEEN-20	0.50ml
Deionized Water q.s.	1,000.00 ml

The paragraph on page 10 beginning at line 5,

Conjugate and Substrate:

Affinity-Purified Anti-Mouse IgG Peroxidase-Labeled conjugate is obtained from Kirkegaard and Perry Laboratories, Inc. (Catalog No. 074-1802). The procedure for determining the optimal dilution of conjugate is detailed below. The Peroxidase Substrate Solutions (ABTS) are obtained from Kirkegaard and Perry, Inc.

Titration of Conjugate: An ~~Immulon~~IMMULON II Flat-Bottom Plate is coated with 100 ul per

well of 20 ug per mL *Mycoplasma hyopneumoniae* Whole Cell Antigen diluted in 10mM GBS. The plate is incubated for no less than one hour at 37°C+2°C and is transferred to 2-7°C for no less than 18 hours and no more than one week. Prior to using the plate is washed three times with PBST, with a one-minute soaking time between each wash, and is tapped to dry. A 1:40 dilution in PBST of the Positive Control Serum is prepared, and the diluted Positive Serum (100 uL/well) is added to one-half of the wells in the plate. PBST is added to the other one-half of the wells. The plate is incubated for one hour at room temperature, after which the plate is washed three times. The conjugated serum is serially diluted with PBST two-fold, starting with a 1:100 dilution and ending with a 1:10,240 dilution. 100 ul of each conjugate dilution is added to four wells of the Positive Serum and four wells of the PBST, and is allowed to react for one-half hour at room temperature. The plates are washed four times, and 100 ul of the Peroxidase Substrate Solution (abts) is added per well. The plate is read at a dual wavelength setting of T λ = 450. A dilution of Conjugate is chosen that gives a reading of 0.850 to 1.050 for the Positive Control Serum when the value of the PBST Control is subtracted from the value of the Positive Control Serum.

The paragraph on page 11 beginning at line 1,

The ELISA is performed as follows: Dynatech ~~Immulon~~IMMULON II Flat-Bottom Microtiter Plates are used. One vial of Lyophilized *Mycoplasma hyopneumoniae* Whole Cell Antigen is reconstituted to ten ml with Glycine Buffered Saline (GBS). The concentration of the reconstituted *Mycoplasma* Protein is 20 µg/mL. 100 µl (2 µg) of diluted antigen is then added to all wells of a plate. The plate is incubated at 37°C $\pm$ 2°C for no less than one hour and then transferred to 2-7°C for a minimum of 18 hours and a maximum of one week. The plates are washed three times with PBST, soaking one minute between each wash, and are then tapped dry. Serums are diluted 1:40 in PBST. The Positive Control Serum will be included in quadruplicate on every plate. Sample volume per well is 100 µl. Serial Test Serum Samples and Reference Serum Samples will be tested in duplicate on the same plate. The plates are incubated for one hour at room temperature, and are washed three times with PBST. 100 µl of Anti-Mouse IgG Peroxidase-Labeled Conjugate (Kirkegaard and Perry), diluted in PBST is added to all wells, and the wells are incubated for 30 minutes at room temperature. The plates are washed four times with PBST. 100 µl of a Peroxidase Substrate Solution (ABTS) is added to all wells, and the plates are incubated until the Positive Serum Control reaches an OD_{405} (450) of 0.850 to 1.050 when the machine is blanked against the PBST Control Wells. The plates are read, and are blanked against the PBST Wells. To be a valid test, the sera of mice inoculated with the Reference Bacterin

must produce a minimum average value of 0.500, and the sera of the non-vaccinated control mice must not exceed the maximum average value of 0.100. Or, the difference between the average value of the sera of mice inoculated with the Reference Bacterin and the sera of the non-vaccinated control mice must be greater than or equal to 0.400.

The paragraph on page 12 beginning at line 7,

The vaccine for testing was prepared according to the procedures detailed in Example 1 using 5% of a mixture of a metabolizable oil that comprises one or more terpene hydrocarbons and a polyoxyethylene-polypropylene block copolymer (Squalane/~~Pluronic~~PLURONIC® L121 mixture) and 0.2% polyacrylic acid polymer (~~Carbopol~~CARBOPOL) as adjuvant, and $2x10^9$ *M. hyopneumoniae* DNA cell equivalents (MHDCE) per dose.

The table on page 20,

Test Vaccine A

Amounts for 1,000,000 doses (2mL each):		% vol/vol
Mycoplasma Concentrate (>1.0 x 10^{10} MHDCE/ml)	1200,000 mL	60.0
Squalane/~~Pluronic~~PLURONIC® L121 mixture	200,000 mL	10.0
~~Carbopol~~CARBOPOL (2% w/v in water)	200,000 mL	10.0
Thimerosal Solution 1% w/v in water and EDTA (tetrasodium salt) 7 w/v%	18,000 mL	0.9
Sterile Saline	382,000 mL	19.1

The pH of the serial is adjusted to 7.0 ± 0.2.
MHDCE = Mycoplasma hyopneumoniae DNA Cell equivalents

The abstract of the invention,